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Form PTO-1390	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER BONN-062
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. 09/936076
INTERNATIONAL APPLICATION NO. PCT/FR00/00579	INTERNATIONAL FILING DATE March 9, 2000	PRIORITY DATE CLAIMED March 9, 1999
TITLE OF INVENTION: DEVICE AND METHOD FOR POSITIONING A LIQUID		DATE: September 7, 2001
APPLICANT(S) FOR DO/EO/US Bruno COLIN and Jacques DACHAUD		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [XX] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. [ ] This a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. [XX] This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [XX] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [XX] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [XX] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).

6. [XX] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [XX] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [XX] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [XX] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [XX] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [XX] Other items or information:

    a. WO 00/53319 (first page only)
    b. International Search Report (PCT/ISA/210)
    c. Written Opinion (PCT/IPEA/408)
    d. International Preliminary Examination Report (PCT/IPEA/409)

U.S. Application No. 09/936076	International Application No. PCT/FR00/00579	Attorney's Docket No. BONN-062

17. [XX] The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO. **$860.00**	860.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482). . **$690.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$710.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . . . **$ 1000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4). . . . . **$ 100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	12 - 20	0	x $ 18.00	$	
Indep.claims	1 - 3	0	x $ 80.00	$	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
			TOTAL OF ABOVE CALCULATIONS =	$ 860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
			SUB TOTAL =	$ 860.00	
Processing fee $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
			TOTAL NATIONAL FEE =	$ 860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
			TOTAL FEES ENCLOSED =	$ 860.00	
				Amount to be: refunded	$
				charged	$

a. [XX] A Credit Card Payment Form in the amount of $ 860.00 to cover the above fee is attached.

b. [ ] Please charge my Deposit Account No. 50-1258 in the amount of $_____ to cover the above fees. Two copies of this sheet are enclosed.

c. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1258 . Two copies of this sheet are enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Registration Number

September 7, 2001
Date

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Bruno COLIN and Jacques DACHAUD

Serial Number: New Patent Application

Filed: September 7, 2001

For: DEVICE AND METHOD FOR POSITIONING A LIQUID

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

September 7, 2001

Please amend this application, prior to calculation of the filing fee, as follows:

IN THE SPECIFICATION:

Page 1, between the title and the first heading, please insert the following:

This application is a U.S. National Stage of International application PCT/FR00/00579, filed March 9, 2000 and published on September 14, 2000 in the French Language.

Page 4, lines 3-9, rewrite the paragraph as follows:

Patent application EP-A-0.803.288 describes, according to one of the embodiments presented, the isolation of a predetermined volume of liquid in order to perform a testing operation at a later stage. This aliquotting is achieved by means of channels with

different diameters and a receiving compartment for the aliquot and an excess of the liquid of which the volume has to be determined.

IN THE CLAIMS:

Please cancel claims 1-12 without prejudice or disclaimer.

Please add new claims 13-24 as follows:

13. (New) A positioning device for a liquid sample, to be displaced by varying the pressure between an intake and an outlet, with a receptacle connecting the intake and the outlet, wherein the displacement of the sample is automatically stopped as soon as said sample has filled the receptacle and reached the point of intersection between a branch connection and said outlet, the branch connection connecting said intake directly to the outlet, and wherein a channel drains the liquid sample in excess.

14. (New) The device of claim 13, wherein the branch connection is connected to the intake of another receptacle, and in that at least two receptacles are arranged in series.

15. (New) The device of claim 13, wherein the cross sectional area along the entire length or any portion of the length of the branch connection is smaller than that of the intake and/or of the outlet.

16. (New) The device of claim 13, wherein the receptacle includes a channel having a diameter which is substantially identical to that of the intake and/or of the outlet.

17. (New) The device of claim 13, wherein the receptacle includes a compartment having a cross-sectional area which is greater than the diameter of the intake and/or of the outlet.

18. (New) The device of claim 13, wherein, between the intake and the branch connection, there is a system capable of bursting any bubbles that might be formed in the liquid sample.

19. (New) The device of claim 18, wherein the bubble-bursting system includes a channel having a cross-sectional area which is significantly greater than that of the branch connections.

20. (New) The device of claim 13, wherein at least one means of drainage for the liquid sample runs longitudinally along either the whole length or part of the length of each channel constituting the intake and/or the outlet and/or each receptacle.

21. (New) The device of claim 13, wherein at least one of the receptacles is associated with a buffer supply.

22. (New) A method for using the device of claim 13 in which at least two receptacles are arranged in series with the following characteristics: a positioning device for a liquid sample to be displaced by a pressure differential between an intake and an outlet, a receptacle connecting intake and outlet, wherein displacement of the sample is automatically stopped as soon as said sample is present in the receptacle and is no longer present at the point of intersection between a branch connection and said intake, with the branch connection connecting said intake directly to said outlet, and the volume of each receptacle being calculated according to the distribution required for each reaction line associated with at least one receptacle.

23. (New) The method of claim 22, wherein the volume of all the receptacles is identical.

24. (New) The method of claim 22, wherein the filling of a receptacle is carried out by gravity.

IN THE ABSTRACT:

Please replace the original abstract with the attached Substitute Abstract.

REMARKS

This Preliminary Amendment cancels claims 1 - 16, adds new claims 17 - 32, amends the specification and presents a new Abstract. The amendments to the specification insert a reference to parent application PCT/FR00/00578 pursuant to 37 C.F.R. § 1.78, and rewrite a paragraph on page 4 to in accordance with the change made during International Preliminary Examination. New claims 17-32 are based on the amended claims presented during International Preliminary Examination, and have been further amended by eliminating multiple dependencies and drawing reference numerals, and by otherwise conforming the claims to U.S. practice. The Substitute Abstract is based on the PCT Abstract. A version with markings to show changes made is attached as an Appendix. Claims 17-32 are pending.

An Information Disclosure Statement is attached.

It is not believed that any fee is required for entry and consideration of this Preliminary Amendment. Nevertheless, the Commissioner is authorized to charge our Deposit Account No. 50-1258 in the amount of any such required fee.

Prompt and favorable examination of the application are earnestly requested.

Respectfully submitted,

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Enclosures:
- Appendix
- Abstract of the Disclosure
- Information Disclosure Statement

APPENDIX

Version With Markings to Show Changes Made

IN THE SPECIFICATION:

The paragraph inserted between the title and the first heading on page 1 is new.

Page 4, lines 3-9, have been rewritten as follows:

Patent application EP-A-0.803.288 describes, according to one of the embodiments presented, the isolation of a predetermined volume of liquid in order to perform[,at a later stage, transfer and/or] a testing [and/or some other] operation at a later stage. This aliquotting is achieved by means of channels with different diameters and a receiving compartment for the aliquot and an excess of the liquid of which the volume has to be determined.

IN THE CLAIMS:

Claims 1-12 have been canceled.

Claims 13-24 are new.

IN THE ABSTRACT:

The Substitute Abstract is new.

ABSTRACT OF THE DISCLOSURE:

Several positioning devices (21) for a liquid sample (2) displaced by pressure variation between an intake (23) and an outlet (24), a container (25) connecting the intake (23) to the outlet (24). In one particularly interesting embodiment, the sample (2) is automatically stopped in its displacement as soon as the sample (2) is present in the container (25), and no longer present at an intersection point between a branch connection (26) and the intake (23), the branch connection (26) directly connecting the intake (23) with the outlet (24). The positioning devices are particularly applicable to microfluidic devices used in biology.

Device and Method for Positioning a Liquid

DESCRIPTION

This invention concerns a device for positioning a fluid sample which is displaced by pressure variation between an intake and an outlet, a receptacle connecting the intake to the outlet.

The background art is given in document EP-A-0.674.009 which describes an apparatus for performing a processing method for a liquid biological sample (e.g. nucleic acid amplification) which includes a well into which the test sample can be introduced and then, once the reaction has occurred, the removal of the sample under the action of a pneumatic chamber. Between these two independent steps, the sample is displaced inside the apparatus in order to, on the one hand, perform decontamination in one chamber and, on the other hand, amplification in a second chamber. The well and the decontamination, amplification and pneumatic chambers are continuous with one another. These different zones are separated by internal partitions inside the apparatus through which passage is only possible via very small channels located in the lower part (which reduces evaporation).

The main problem with this type of transfer resides in the fact that there is a fluid continuity between all compartments. Therefore, there is no clear physical separation between pairs of compartments which might be very different, e.g. the decontamination and amplification chambers. As a result, there is

a risk that nucleic acids (normally purified) should remain contaminated, which would compromise the amplification step. Moreover, during pressure-mediated transfer of the liquid between the first two compartments, the liquid might continue traveling over into another chamber, thereby affecting the accuracy of the analysis.

Document WO-A-97/21090 also uses very small channels between different chambers in a disk-shaped device. This device is provided with an axis of rotation at its center. Liquid movements are controlled by: firstly, centrifugal force for displacement of the liquids in a main channel that links two distribution chambers; and secondly, centripetal acceleration for displacement of the liquid contained in a distribution chamber towards an analysis chamber via a secondary channel. These channels are fitted with valves that either allow or block passage of the fluid.

In this case, the valves enable the various chambers to be more effectively separated. Of course, this technique requires the installation of many valves in order to set up several parallel reaction lines. An actuating mechanism must be associated with each valve. Therefore, the cost of this technology is relatively high.

Finally, document WO-A-98/07019 has a similar structure to the preceding one but with two key differences. Firstly, displacements

are only driven by centrifugal force. Secondly, there are no valves. Thus, centrifugal displacement of the liquid is controlled by the presence of very small channels between the different reservoirs and by capillary force. In order to overcome this capillary force so that transfer can occur, the centrifugal force must be relatively strong.

In this case, the main problem lies in having to control centrifugation very precisely. Thus, the absence of valves and the arrangement of numerous receptacles in series according to the direction of the centrifugal force means that the centrifugation has to be strong enough to drive the liquid from receptacle N towards adjacent, outer receptacle N+1, but weak enough to prevent the liquid from being transferred from receptacle N to a farther receptacle N+2 or N+n, where n is equal to or greater than 3.

Moreover, for the latter document as for the preceding one, centrifugation entails another problem. It is impossible to carry out chronologically different liquid transfer operations in two parallel reaction lines on the same apparatus since all liquids have to be at the same level for a given centrifugation operation. If this were not the case, control would have to be even more precise and the reaction lines would have to be offset from one another with respect to the center of rotation. This would make using it difficult and would introduce imbalance during

centrifugation by virtue of the uneven distribution of the various masses.

Patent application EP-A-0.803.288 describes, according to one of the embodiments presented, the isolation of a predetermined volume of liquid in order to perform, at a later stage, transfer and/or testing and/or some other operation. This aliquotting is achieved by means of channels with different diameters and a receiving compartment for the aliquot and an excess of the liquid of which the volume has to be determined.

This invention makes it possible, according to its method, to isolate this type of aliquot, but this can be done in series or even in parallel in several different compartments. Moreover, the device according to the invention allows for highly accurate positioning of an exact volume of transferred liquid. This is particularly valuable in biological test sample cards inside which very small volumes of liquid have to be transferred.

In accordance with this invention, the device makes it possible to know exactly where a volume of liquid being displaced is within a network of channels inside an apparatus wherein at least one biological reaction can be performed.

To this end, this invention concerns a device for positioning a liquid sample which is displaced by means of a pressure variation between an intake and an outlet, a receptacle connecting the intake

to the outlet, characterized in that displacement of the sample is automatically stopped as soon as said sample has filled the receptacle and reached the point of intersection between a branch connection and said outlet, the branch connection also directly connecting said intake and outlet.

In such a case, the branch connection is connected to the inlet of another receptacle, and the number of receptacles arranged in series is at least two. Filling of a receptacle is by means of gravity.

The pressure differentials used in this device are small (e.g. lower than 300 millibar and advantageously below 100 millibar for liquid displacement), a fact which entails a number of advantages when it comes to implementation. Thus, the cost of the pump device meant for producing the pressure differential is reduced. The requirements on material and components in terms of dimensioning precision, behavior under pressure and assembly accuracy are lowered, which would contribute to a significant reduction in cost. The cross-section of the entire length or part of the length of the branch connection is smaller than that of the intake and/or the outlet.

According to a first modified embodiment, the receptacle consists of a channel with a diameter which is substantially

identical to or greater than the diameter of the intake and/or the outlet.

According to a second modified embodiment, the receptacle consists of a compartment with a cross-sectional area greater than the diameter of the intake and/or the outlet.

According to a third modified embodiment, a means of bursting any bubbles which might form in the liquid sample is included between the intake and the branch connection.

In this case, the bubble bursting system consists of a channel which has a significantly larger cross-section than that of the branch connection.

According to particular embodiment, a strip to promote drainage of the liquid sample runs along the whole length or part of the length of at least one channel, corresponding to the intake and/or the outlet and/or each receptacle.

According to another embodiment, at least one of the receptacles is associated with a buffer supply. Such a buffer supply is thoroughly described and protected in the patent application filed by the applicant on the same day as this invention and entitled: "Test sample card with improved filling". In order to provide a comprehensive description, the contents of the description of this patent application are considered as being included in this invention.

The invention also concerns a method for using a device, such as a device for positioning a liquid sample, in which the liquid is displaced by means of a pressure differential between an intake and an outlet, a receptacle connecting intake and outlet, characterized in that displacement of the sample is automatically stopped as soon as said sample is present in the receptacle and no longer present at the point of intersection between a branch connection and said intake, the branch connection directly connecting said intake and outlet. In this method, at least two receptacles are aranged in series and the volume of each receptacle is calculated on the basis of the distribution desired for each reaction line associated with at least one receptacle.

Preferably, all the receptacles have exactly the same volume.

Such an apparatus can be used for the analysis of one or more different liquid samples to identify one or more analytes, using any method, be it a simple or complex method and be it based on one or more different reagents, depending on the chemical, physical or biological nature of the analyte being tested. The technical principles defined hereafter are not restricted to any single, specific analyte; the only required condition being that the analyte must either be dissolved or in suspension in the test sample. In particular, the test process being used can be performed on a homogenous, heterogeneous or mixed form.

One particular application, not limited to such a device, concerns biological tests for the detection and/or quantitative determination of one or more ligands, in which the assay involves one or more anti-ligands. The word ligand is taken to mean any biological species, e.g. an antigen, a fragment of an antigen, a hapten, a nucleic acid molecule, a fragment of nucleic acid, a hormone or a vitamin. One example of an application of the test methods concerns immunoassays, whatever their particulars and whether the assay is direct or based on competition. Another example of an application concerns the detection and/or quantitative determination of nucleic acids, including all operations required for such detection and/or quantitation in any kind of sample containing the target nucleic acid species. Among such diverse operations, the following could be specified: lysis, melting, concentration, enzyme-mediated nucleic acid amplification, and any detection modalities which include a hybridization step using, for example, a DNA chip or a labeled probe. Patent application WO-A-97/02357 stipulates the various stages involved in the case of nucleic acid analysis.

The accompanying drawings are given by way of example and are not to be taken as in any way limiting. They are intended to make the invention easier to understand.

Figure 1 shows a diagrammatic view of a first embodiment of the device according to the invention.

Figure 2 shows a diagrammatic view of a second embodiment of the device according to the invention.

Figure 3 shows a number of the devices as described in Figure 2, arranged in series and in parallel.

Figure 4 shows a cross-sectional view through A-A in Figure 3.

Figure 5 shows a diagrammatic view of a third embodiment of the device according to the invention.

Figure 6 is a diagrammatic view according to Figure 5 showing the first stage in the positioning of a liquid sample, according to this invention.

Figure 7 is a diagrammatic view according to Figure 5 showing the second stage in the positioning of a liquid sample, according to this invention.

Figure 8 is a diagrammatic view according to Figure 5 showing the third stage in the positioning of a liquid sample, according to this invention.

Figure 9 shows a number of different devices arranged in series, according to a fifth embodiment of the invention.

Figure 10 shows a detailed view of the device shown in Figure 9.

Figure 11 shows a cross-sectional view through B-B in Figure 10 in which the means of directing the liquid is apparent.

Finally, Figure 12 shows a cross-sectional view through C-C in Figure 5.

This invention concerns four positioning devices which can be used to position a liquid sample (2) as will be fully explained with reference to the accompanying drawings.

The first embodiment is shown in Figure 1. It concerns a positioning device (1) according to an embodiment which essentially comprises a channel with the same cross-sectional area throughout its length. This channel effectively includes three, functionally distinct zones at the level of the positioning device (1).

First of all, there is an intake (3) via which the liquid sample (2) can be introduced into the positioning device (1). Next there is a receptacle (5) which is continuous with the intake (3) and which receives and makes it possible to position said liquid sample (2). This receptacle (5) has a curved configuration. Finally, there is an outlet (4) present in the prolongation of the intake (3) and the receptacle (5) which makes it possible to drain the sample (2) out of the device.

Moreover, the channel is associated with a branch connection (6) in a substantially parallel arrangement, similarly connecting intake (3) and outlet (4). According to a preferred embodiment,

the cross-sectional area and diameter of this branch channel are substantially smaller than the cross-sectional area and diameter of the channel which makes up the intake (3), the outlet (4) and the receptacle (5). The difference in diameter may be anything from two-fold to five-fold. The shape and diameter of the various channels can also be varied according to the nature of the liquids to be transferred. For wetting liquids such as an aqueous solutions containing Triton X100 [registered trademark] or Tween [registered trademark] in proportions of between 0.5 and 2ml/l, it is important to prevent capillary action which would cause the liquid to rise back up inside the branch connection (6) thereby causing a blockage and preventing the passage of air through it. This problem will be resolved by those skilled in the art who will determine the appropriate cross-sectional area based on the specific liquid to be used and the materials the device is made of. With non wetting liquids such as distilled water, capillary action does not occur.

Although there are certain structural differences, the other three embodiments shown in Figures 2 to 11 are relatively close to this structure.

Thus, a second embodiment (11) is shown in Figure 2, in which there are two differences with respect to the first embodiment shown in Figure 1. Firstly, it can be seen that the channel is

perfectly straight, i.e. the intake (13), the receptacle (15) and the outlet (14) are in line. Of course, this embodiment is not compulsory and it would be entirely possible to use a curved receptacle (5) as shown in Figure 1. Secondly, the point of intersection between the intake (13) and the branch connection (16) includes a system (18) for bursting any bubbles which might form in the liquid sample (2) while it is being transferred inside the main channel (13-14-15). Therefore, this device (11) is structurally identical but the way in which these two different embodiments work will be more better explained in Figures 5 to 8 below.

Thus, the third embodiment is shown in Figures 5 to 8. This device (21) shares its main characteristics with the first two embodiments, i.e. it contains an intake (23), an outlet (24) and a receptacle (25), the receptacle (25) having a curved shape like that in receptacle 5 in the first embodiment. Similarly, the branch connection (26) still connects intake (23) and outlet (24). However, as in the embodiment in Figure 2, a bubble-bursting system is included between the intake (23) and the branch connection (26). No liquid sample (2) is shown in Figure 5.

Figure 11 shows a cross section through C-C in Figure 5 which reveals the special arrangement of the channels at the point of intersection between the intake (23) and the branch connection (26) when a bubble-bursting system (28) is present. The depth of the

channels is such that an angle is formed between 23 and 28, and another between 28 and 26. That between 23 and 28 creates a sharp edge which enhances bubble-bursting efficacy. Preferably, there also exists another sharp edge between 26 and 28.

As described below by way of example and using a ball drill to machine the device in a plastic card made of impact polystyrene, channel 23 could be made with a depth of 2 millimeters (mm) and a width of 2 mm, the bubble-bursting system (28) could have a depth of 1.5 mm and a width of 3 mm, and the branch connection (26) a depth of 0.5 mm and a width of 0.5 mm. In these conditions, if 100 microliters of distilled water are being displaced by a pressure differential at a rate of 50 microliters per minute, the liquid will fill the cavity (25) and then displacement will stop. In this embodiment, the volume of liquid to be isolated must be smaller than the volume of the receptacle (25).

From Figures 6 to 8, it is easier to understand how this device (21) works, a mode of operation which is identical to those of the first two embodiments. Thus, as can be seen in Figure 6, the liquid (2) arrives at the intake (23) and, as a result of the application of pressure from outside (P in Figure 7), the liquid (2) is driven into the receptacle (25). It is preferable to use gravity to drive such a movement (in direction F1). Thus, the dimensions of the branch connection (26) and of the channel

comprising the intake (23), the outlet (24) and the receptacle (25) can be calculated to insure that the liquid (2) is spontaneously directed towards the receptacle (25) by the pressure (P). This is clearly shown in Figure 7 in which, under the influence of pressure P, all the liquid sample (2) is pushed into the receptacle (25) and is displaced in the direction of the arrow F1 towards the outlet (24). Of course, the volume of liquid which is pushed by pressure P is less than or equal to the volume contained in the receptacle (25), i.e. less than or equal to the volume located between the two points of intersection between the branch connection (26) and, on the one hand, the intake (23) and, on the other hand, the outlet (24). From Figure 8, it is easier to understand the real function of the positioning device (21) and of the branch connection (26). Thus, the liquid sample (2) is all found inside the receptacle (25), confined between the intake (23) and the outlet (24). In this case, pressure P is still present but the pushing force is no longer directed into the liquid but around the branch connection in the direction of the arrows (F2). As a result, the liquid sample (2) is indeed positioned at the desired spot. Another channel, an outlet channel (53), could be included in the receptacle (25) which would allow (depending on whether a valve not shown in the Figure were open or closed) transfer of the known volume of sample (2) from this predetermined position to a receptacle in which a

biological reaction could be carried out, e.g nucleic acid amplification, antigen-antibody binding, etc..

In order to drive this transfer, it is possible to use a pumping device which is fully described and protected in the patent application entitled "Pumping device for transferring at least one fluid into a sealed consumable" submitted by the applicant on the same day as this invention. The contents of the description of this patent application are considered as being included in this invention. A valve system which could be used in the device described in this patent application has already been described in a patent application submitted by the applicant on September 8 1998 under application number FR98/11383 and entitled: "A device in which reactions can be performed, a transfer system between devices and a method for using such a system". The contents of the description of this patent application are considered as being included in this invention.

Nevertheless, it is still possible to move the liquid (2) by increasing the pressure P applied to it when it is present in the receptacle (25).

In all these examples, it is obvious that the embodiment exploits a pressure (P) applied at the intake of the receptacle (25) although the system would function in the same way if a vacuum

(D) were applied at the outlet of the receptacle (25). Alternatively a combination of both could be used.

These different positioning devices (1, 11, 21) together with a fourth device (31) which has not yet been described are perfectly suited to use in a card (40) which is clearly shown in Figure 3. This card (40) makes it possible to conduct multiple biological reactions inside a sealed consumable without any action necessary inside the card (40). Thus, it is rather by virtue of an action conducted from the outside of said card (40) that liquid samples (2) are directed from one receptacle (5, 15 or 25) to another receptacle (5, 15 or 25) for the purposes of either storing them, transferring them at a later stage or mixing them with another sample, be it liquid or solid, present in the compartment.

Figure 3 shows three reaction lines (50), each comprising two positioning devices (11) as shown in Figure 2. It can be seen that, between the intake (54) and the outlet (55), there are valves (51) which can be used to either open or close channels for transfer of the liquid samples (2) from one receptacle (15) to another receptacle (15) or to the outlet (55). As will be explained later on, the presence of these valves is not compulsory. In a particular embodiment of any of the devices described, in which one compartment has to be heated (to any temperature between 30 and 120 °C and advantageously to between 40 and 95 °C) to favor

some chemical or biological reaction, the presence of the valves will inhibit evaporation phenomena which might affect the volume inside the compartment and thereby lead to unregulated heating of the reagents or problems of cross-contamination between different compartments.

In this case, it might be advantageous to position extra valves on the branch connections.

Figure 4 is a cross-sectional view through A-A in Figure 3 and shows that the two receptacles (15) arranged in line can be positioned on different sides of the card (40). Thus, the first receptacle (15) shown on the left opens onto the upper side whereas the second receptacle (15) shown on the right opens onto the lower side. Of course, to allow transfer of the samples (2), a film has to be stuck onto either side of the card (40); these films are marked as 56 on both sides of said card (40).

The nature of the flexible film may vary according to the nature of the test card and of the fluids being tested, especially when compatibility is at issue. For example, TPX (polymethyl pentene copolymer) or BOPP (bi-oriented polypropylene) films are suitable for biological assays. These films can be fixed in place either using an adhesive, (with the adhesive applied to the film, e.g. a silicon-based adhesive) or by heat-sealing. An example of

a BOPP adhesive is available from BioMérieux Inc. (St. Louis, MO, USA) (reference: 022004-2184).

In terms of production, the card is manufactured by the machining of special plastic material, e.g. impact polystyrene (reference: R540E from the Goodfellow company) which is compatible with the liquids being processed. For industrial-scale production, the card could be manufactured by precision molding, but any other manufacturing method (including those used in the semi-conductor industry as stipulated in patent application WO-A-97/02357) may be used for test card production.

Therefore, each card (40) can include several positioning devices (1, 11 or 21) arranged in series, following one another. It is also possible to have reaction lines (50) comprising several sets of devices in series all arranged in parallel, as clearly shown in Figure 3. According to a modified embodiment, the various different embodiments of the positioning device can be combined, as shown below. Finally, it is also possible to vary the volume of each receptacle (5, 15 or 25) in order to vary the volumes transferred according to the reactions to be performed subsequently.

Thus, when the sample to be isolated, in order to be able to displace it subsequently, is to have a large volume, a receptacle of a volume corresponding to the first and third embodiment would

be selected whereas, for a smaller volume (with channels having the same cross-sectional area), it would be necessary to use the second embodiment in Figure 2. The volume of the sample (2) can also be varied by modifying the length and/or the diameter of the receptacle (5, 15 or 25). All the possible alternatives above can also take into account the device 31 according to the fourth embodiment.

This fourth embodiment is clearly shown in Figures 9 to 11.

In Figure 9, it can be seen that there is a reaction line (52) which is substantially identical to the reaction lines (50) represented in Figures 3 and 4. One of the devices (31) which make up this reaction line is, as for it, better represented in Figure 10. As in the first three embodiments, there is an intake (33) and an outlet (34).

There are a number of novel features in this embodiment, including the volume of the receptacle (35) which is not made up of a channel as was the case previously but rather of a compartment, the volume of which is significantly greater. This embodiment is more suitable for a card in a substantially vertical position so that filling of the receptacle (35) is made easier by gravity. Another major difference is the presence of a branch connection consisting of two dissimilar parts (36 and 37). The first portion (36) intersects the intake (33) and the second portion (37)

intersects the outlet (34). Of course, the two parts of the branch connection are connected to one another at a point of intersection from which issues a channel (57) for draining any excess liquid sample (2). According to a preferred embodiment, this channel has a diameter similar to that of the first portion of the branch connection (36) and is arranged in line with it.

It is also possible to associate the receptacle (35) with a buffer supply as described previously.

The filling method in this fourth embodiment is therefore substantially different from that used for the three embodiments described previously. Now referring to Figure 9, it can be seen that a liquid sample coming from the left hand side is going to be introduced into the first device (31) and is going to fill the receptacle or compartment 35 up to the point of intersection between the outlet (34) and the second portion of the branch connection (37). As soon as the liquid reaches this point of intersection, a far greater force is needed to drive the liquid (2) into the second portion of the branch connection (37) compared with that required to drain the sample via the first portion of the branch connection (36). As a result, any excess of said sample (2) will pass through the drainage channel (57) via the first portion of the branch connection (36) and will be able to fill the second receptacle (35) which follows it. In this way, by inducing a

certain rise in pressure in a compartment containing said liquid sample (2), the volume of which is greater than that of all the compartments (35), it is thus possible to transfer a liquid sample (2) into various positioning devices (31) and thereby obtain equal aliquots, i.e. an identical volume in each of said receptacles (35). It can also be seen that, since the diameter of the first portion of the branch connection (36) is substantially identical to that of the channel which makes up the intake (33), this first portion (36) constitutes a bubble-bursting system (38) at the point of intersection between itself and said intake (33).

Unequal aliquots can also be generated if the compartments (35) are of different volumes, depending on the use to which each compartment (35) is to be put in the reaction. In one embodiment, the volume of the sample to be aliquotted is equal to the total volume of compartments (35). In another embodiment, the volume of the sample to be aliquotted is greater than the entire volume of all the compartments (35). In this case, the upper lines (35 and 36) can be drained by applying a pressure differential or some other means, or alternatively a special compartment (35) could be built in at the end of the reaction line (52) to act as a receiving compartment for any excess liquid.

As previously stipulated, different embodiments of this positioning device (1, 11, 21 and 31) can be combined, as

represented below. It is also possible to vary the volume of each receptacle (5, 15, 25 or 35) in order to control the volumes being transferred according to the reactions which are to be carried out subsequently.

Thus, when the test to be carried out on the isolated sample can be performed using the entire volume of the sample, one would tend to choose one of the first three embodiments. When the test to be carried out involves aliquotting the sample – be it the division of the sample into equal or disparate volumes in different compartments – one would tend to prefer the fourth embodiment. This applies, for example, when a sample is being subjected to multiple tests as in diagnostic testing for several different pathogenic agents either by immunoassay or by nucleic acid hybridization techniques. This device can be used to isolate volumes of liquid of between 1 and 5000 microliters (advantageously of between 5 and 2000 microliters and preferably of between 10 and 1000 microliters).

It can also be seen that the intake (33) extends from the receptacle (35) with a shape which is particularly apparent in the cross-sectional view shown through B-B in Figure 11. Thus, the dimensions of the intake (33) are completely normal but, between this intake (33) and the receptacle (35), there is a means of drainage (39) for the liquid sample (2) designed for directing it

towards and facilitating its transfer into said receptacle (35). This means of drainage (39) is obtained by a bevel shape which is significantly farther from the external film (56) which partitions the device (31) at the level of the receptacle than it is at the level of intake (33). The relatively small distance between the means of drainage (39) and the film (56) means that the liquid sample (2) can be more easily directed using simple capillary action.

As in Figure 5, an outlet channel (53) could be built into the bottom of the receptacle (35) to transfer the sample positioned in said receptacle (35) to another receptacle in order to subject it to a reaction or some other kind of manipulation or simply to store it.

REFERENCES

1. Positioning device according to the first embodiment
2. Liquid sample
3. Intake of the receptacle (5)
4. Outlet of the receptacle (5)
5. Receptacle
6. Branch connection between the intake (3) and the outlet (4)
11. Positioning device according to the second embodiment
13. Intake of the receptacle (15)
14. Outlet of the receptacle (15)
15. Receptacle
16. Branch connection between the intake (13) and the outlet (14)
18. Bubble-bursting system
21. Positioning device according to the third embodiment
23. Intake of the receptacle (25)
24. Outlet of the receptacle (25)
25. Receptacle
26. Branch connection between the intake (23) and the outlet (24)
28. Bubble-bursting system
31. Positioning device according to the second embodiment
33. Intake of the receptacle (35)
34. Outlet of the receptacle (35)

35. Receptacle

36. First portion of the branch connection between the intake (33) and the outlet (34)

37. Second portion of the branch connection between the intake (33) and the outlet (34)

38. Bubble-bursting system

39. Means of drainage for the sample (2)

40. Card with several lines (50) arranged in parallel

50. Reaction line comprising several devices (11) arranged in series

51. Valve between two different devices (11)

52. Reaction line comprising several devices (31) arranged in series

53. Outlet channel

54. Intake for a reaction line (50)

55. Outlet for a reaction line (50)

56. Film

57. Channel for drainage of any excess sample

D. Drop in pressure induced to displace the liquid sample (2)

P. Rise in pressure induced to displace the liquid sample (2)

F1. Displacement of the liquid sample (2) under the influence of pressure P

F2. Displacement of the air in the branch connection (26) under the influence of pressure P

CLAIMS

1. A positioning device (31) for a liquid sample (2), to be displaced by varying the pressure between an intake (33) and an outlet (34), with a receptacle (35) connecting the intake (33) and the outlet (34), characterized in that the displacement of sample (2) is automatically stopped as soon as said sample (2) has filled the receptacle (35) and reached the point of intersection between a branch connection (36 and 37) and said outlet (34), the branch connection (36 and 37) connecting said intake (33) directly to the outlet (34).

2. The device, according to claim 1, characterized in that the branch connection (6, 16, 26 or 36 and 37) is connected to the intake (3, 13, 23 or 33) of another receptacle (5, 15, 25 or 35), and in that at least two receptacles (5, 15, 25 and/or 35) are arranged in series.

3. The device, according to either of claims 1 or 2, characterized in that a receptacle (5, 15, 25 or 35) is filled by gravity.

4. The device, according to any of claims 1 through 3, characterized in that the cross sectional area along the entire length or any portion of the length of the branch connection (6, 16, 26 or 36 and 37) is smaller than that of the intake (3, 13, 23 or 33) and/or of the outlet (4, 14, 24 or 34).

5. The device, according to any of claims 1 through 4, characterized in that the receptacle (5, 15 or 25) consists of a channel with a diameter which is substantially identical to that of the intake (3, 13 or 23) and/or of the outlet (4, 14 or 24).

6. The device, according to any of claims 1 through 4, characterized in that the receptacle (35) consists of a compartment, the cross-sectional area of which is greater than the diameter of the intake (33) and/or of the outlet (34).

7. The device, according to any of claims 1 through 6, characterized in that, between the intake (13, 23 or 33) and the branch connection (16, 26 or 36 and 37), there is a system (18, 28 or 38) capable of bursting any bubbles that might be formed in the liquid sample.

8. The device, according to claim 7, characterized in that the bubble-bursting system (18, 28 or 38) consists of a channel, the cross-sectional area of which is significantly greater than that of the branch connection (16, 26 or 36 and 37).

9. The device, according to any of claims 1 through 8, characterized in that at least one means of drainage (39) for the liquid sample (2) runs longitudinally along either the whole length or part of the length of each channel constituting the intake (3, 13, 23 or 33) and/or the outlet (4, 14, 24 or 34) and/or each receptacle (5, 15, 25 or 35).

10. The device, according to any of claims 1 through 9, characterized in that at least one of the receptacles (5, 15, 25 or 35) is associated with a buffer supply.

11. The method for using the device, according to any of claims 1 through 11, in which at least two receptacles are arranged in series with the following characteristics: a positioning device (1, 11 or 21) for a liquid sample (2) to be displaced by a pressure differential between an intake (3, 13 or 23) and an outlet (4, 14 or 24), a receptacle (5, 15 or 25) connecting intake (3, 13 or 23) and outlet (4, 14 or 24), characterized in that displacement of the

sample (2) is automatically stopped as soon as said sample (2) is present in the receptacle and is no longer present at the point of intersection between a branch connection (6, 16 or 26) and said intake (3, 13 or 23), with the branch connection (6, 16 or 26) connecting said intake (3, 13 or 23) directly to said outlet (4, 14 or 24), and the volume of each receptacle being calculated according to the distribution required for each reaction line associated with at least one receptacle.

12. The method, according to claim 11, characterized in that the volume of all the receptacles is identical.

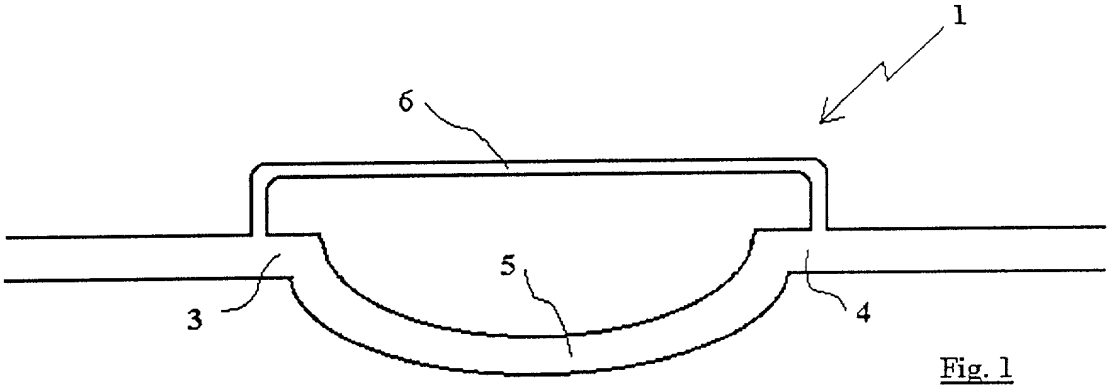

Fig. 1

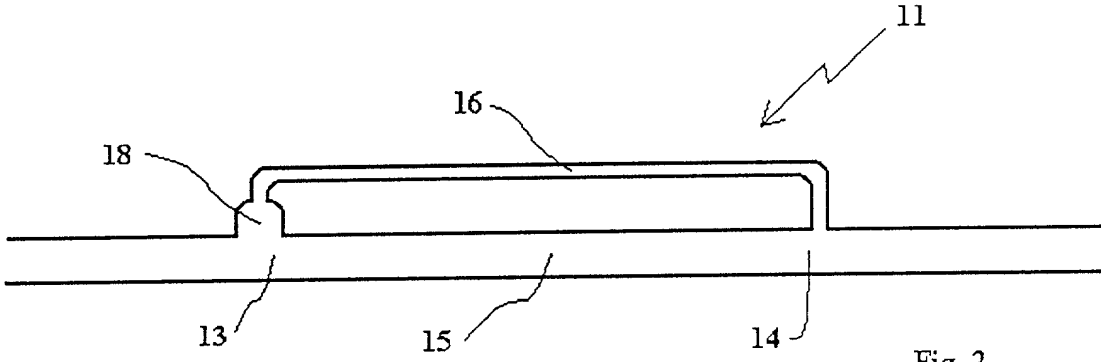

Fig. 2

Section C-C

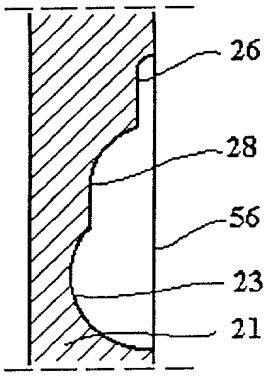

Fig. 12

Section A-A

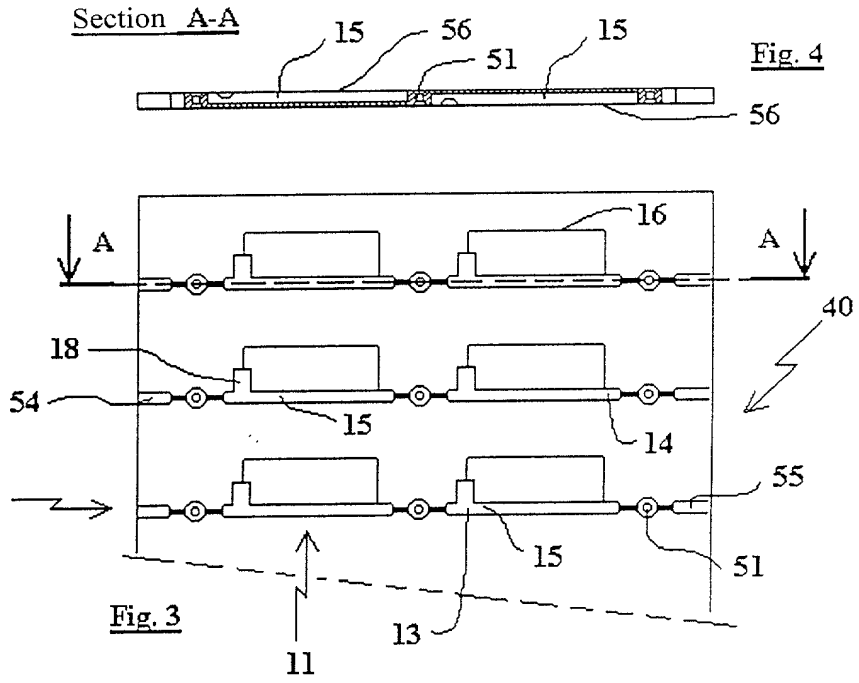

Fig. 3

Fig. 4

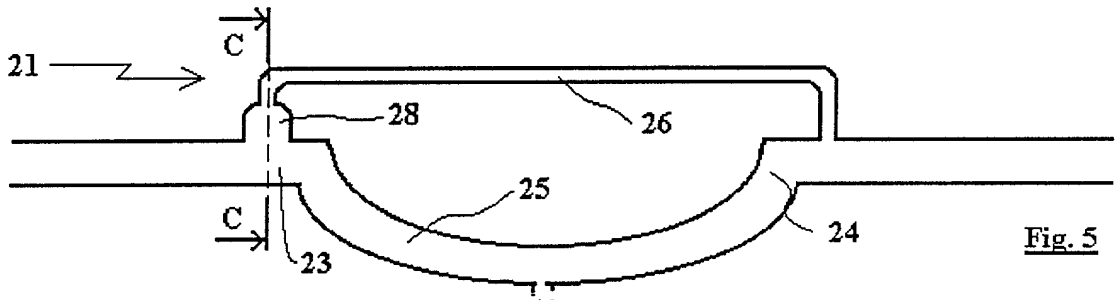

Fig. 5

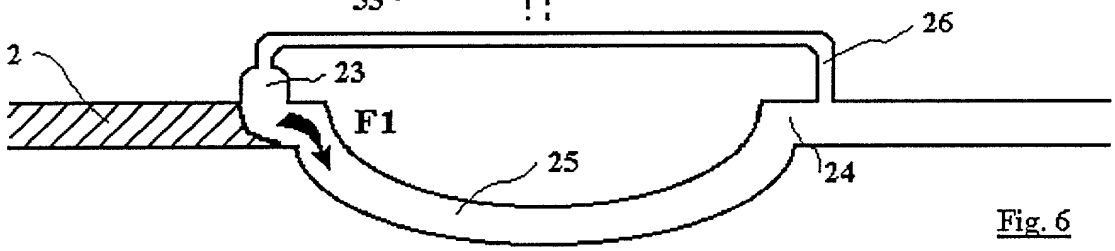

Fig. 6

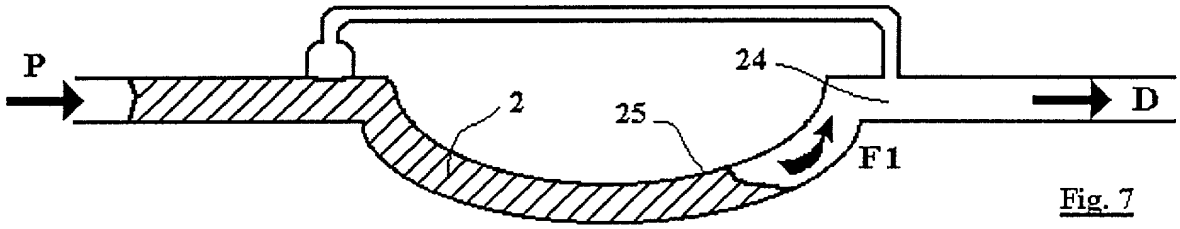

Fig. 7

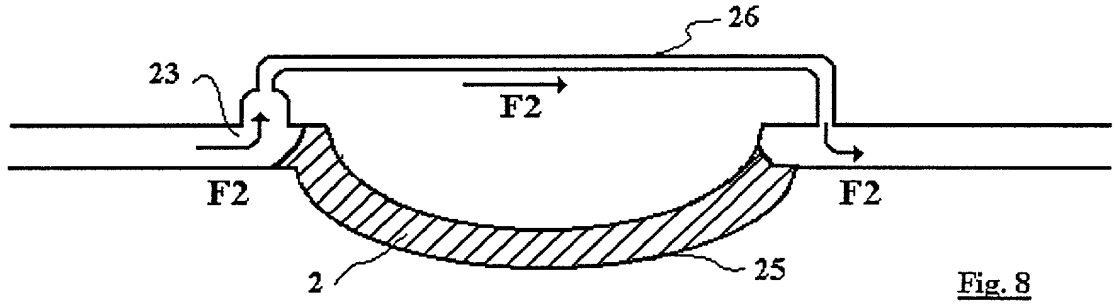

Fig. 8

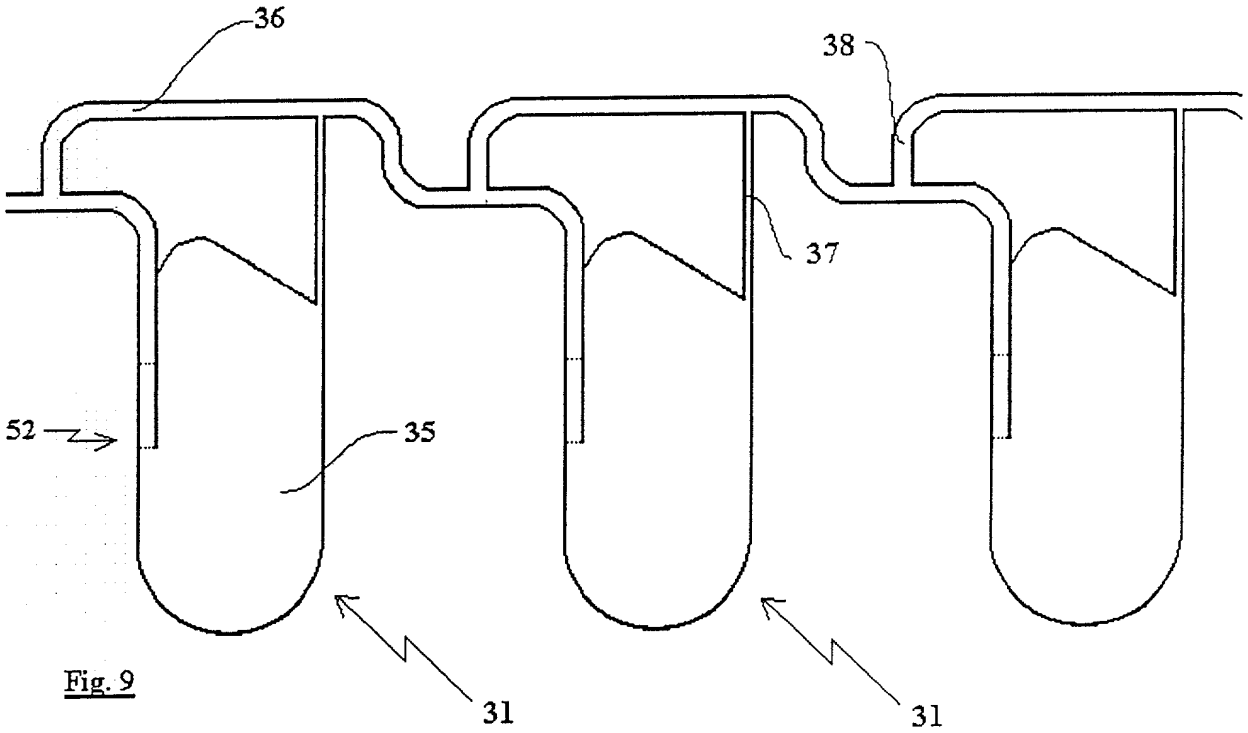

Fig. 9

Section B-B

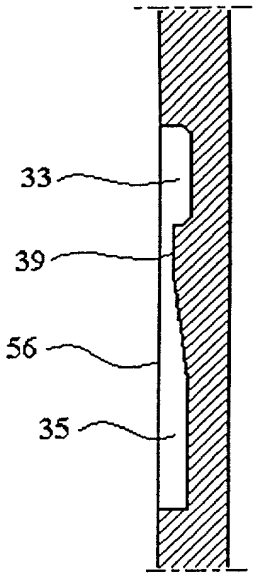

Fig. 11

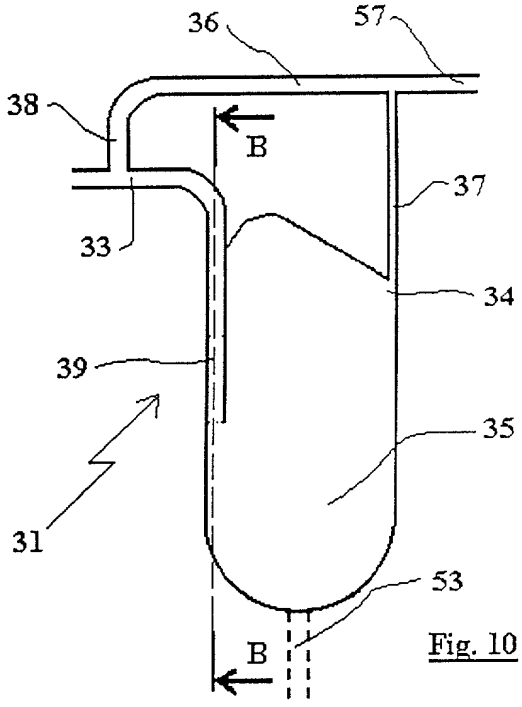

Fig. 10

Docket No. BONN-062

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled (INSERT TITLE) Device and method for positioning a liquid

the specification of which

(Check one of 1, 2, or 3.)

1. __ is attached hereto.
2. XX was filed on March , 9, 2000 as International PCT Application Serial No.PCT/FR00/00579 and was amended on March 13, 2001 (if applicable).
3. __ was filed on _____ as U.S. Application Serial No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application for which priority is claimed:

(List prior foreign applications.)	(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
	99/03033	FRANCE	March 9, 1999	X Yes ___ No
				___ Yes ___ No

___ See attached list for additional prior foreign applications

I hereby claim the benefit under Title 35, United States Code, §120, of any United States application listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56, which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)
(Application Serial No.)	(Filing Date)	(Status)

I hereby appoint as principal attorney James C. Lydon, Reg. No. 30,082.

Please direct all communications to the following address:

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Facsimile: (703) 838-0447

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's Signature: Jacques Dachaud Date: 3.09-2001

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Inventor's Signature: _____ Date:

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Citizenship:

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Residence:

Citizenship:

Post Office Address:

Full name of fifth inventor:

Inventor's Signature: _____ Date:

Residence:

Citizenship:

Post Office Address:

Full name of sixth inventor:

Inventor's Signature: _____ Date:

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Post Office Address:

Full name of seventh inventor:

Inventor's Signature: _____ Date:

Residence:

Citizenship:

Post Office Address:

Full name of eighth inventor:

Inventor's Signature: _____ Date:

Residence:

Citizenship:

Post Office Address: